



Prevalence of Chickpea Wilt in Jammu Sub-Tropics

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Abstract: Fortnightly surveys of different chickpea growing areas of Jammu sub-tropics revealed that overall disease incidence of 15.64 and 16.86 per cent, during *Rabi* seasons of 2016-17 and 2017-18, respectively. *Fusarium oxysporum* f. sp. *ciceri* was isolated from wilt infected chickpea plants and soil samples collected from chickpea fields of Jammu sub-tropics. Pathogenicity test of different isolates of *Foc* exhibited disease incidence (6.26-66.65%) in susceptible cultivar (C-235). Wilt symptoms in adult plants were quite common at flowering and pod stages. The affected plants showed characteristic wilting viz., drooping of the petioles, rachis and leaflets.

Keywords: Prevalence, Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, Pathogenicity

Pulses, being legume crops play a vital role in improving soil fertility and conserve natural resources which are essential for sustainable agriculture. Chickpea (*Cicer arietinum* L.) a self-pollinating diploid ($2n=2x=16$) crop is the world third most important legume. India is a main chickpea producing country followed by Pakistan and Turkey (FAOSTAT 2007). The chick pea production in India has gone up from 38.55 to 112.29 lakh tonnes during 2000-01 to 2017-18, while the area has also gone up from 51.85 to 105.61 lakh ha, whereas, the yield has steadily increased from 744 kg/ha to 1063kg/ha during the same period (Samriti et al 2020). The pathogen of chickpea wilt disease is seed-borne (Pande et al 2007) as well as soil borne nature (Jimenez- Fernandez et al 2011). It can survive in soil for more than 6 months in the absence of its host and can cause severe damage to crop yield and disease can appear at any stage of the plant growth. Early wilting is reported to cause more loss than late wilting, but seeds from late wilted plants are lighter, rougher, and duller than those from healthy plants.

Hence, it is most important to carry out screening of chickpea genotypes under artificial inoculated conditions to identify sources of resistance. In order to develop the disease resistant and high yielding cultivars, it is essential to study and understand the variability in the pathogen. Therefore, integrated management strategies are the possible solutions to maintain plant health mainly for soil borne plant pathogens. These strategies include modification of cultural practices, growing of resistant varieties with minimum application of chemicals (Bendre and Barhate, 1998), and encouragement of beneficial microbial population to reduce pathogen inoculation. Keeping in view the importance of the crop,

losses caused to it and lack of information regarding the status of the disease in Jammu subtropics.

MATERIAL AND METHODS

The field experiments of the present investigation on wilt of chickpea were conducted at Research Farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha situated at 32.43° N latitude, 74.54° E longitude and 327 meters above sea level during *Rabi* 2016-17 and 2017-18 cropping seasons. The laboratory experiments were conducted in the Division of Plant Pathology, SKUAST- Jammu. Materials used and methodology adopted for field as well as laboratory experimentation are described as below:

Field survey of chickpea wilt: Fortnightly field surveys for recording disease incidence were during *Rabi* 2016-17 and 2017-18 crop seasons conducted in chickpea growing areas of Jammu subtropics viz., Jammu, Samba and Kathua districts. In each district, the major chickpea growing blocks were identified, three villages per block and five fields per village were further selected for recording disease incidence on chickpea plants. For recording the observations from each measuring 5m² area were randomly selected and ten plants were tagged for recording disease incidence and average disease incidence in the village was recorded. The per cent disease incidence on infected plants due to chickpea wilt was calculated

Isolation and identification of pathogen associated with chickpea wilt: During the field surveys, diseased chickpea plants with wilt symptom were collected from different districts were carefully uprooted and bagged separately in

perforated polythene bags and brought to the laboratory. Roots of infected chickpea plants were longitudinally cut with a sharp and sterilized blade into two halves from the centre. The infected roots were cut into small bits of 2-3 mm size surface sterilized with sodium hypochlorite (4%) solution for 30 seconds, rinsed thrice with sterile distilled water, blotted dry, transferred to sterilized PDA plates, incubated in BOD incubator at $28\pm 1^\circ\text{C}$ for 7-10 days and allowed the fungal pathogen to grow. Colony characteristics were studied and measurements recorded daily until the growth was static. Pure fungal colonies which developed from the diseased bits were transferred to PDA slants and incubated at room temperature for 15 days. Pure culture of the fungus was maintained at 4°C for further studies.

Symptomatology of fusarium wilt in chickpea: The symptoms of wilt were observed at two different stages. At seedling stage, diseases were observed three weeks after sowing of chickpea plants. Symptoms were expressed as wilting of leaves followed by drooping of leaves and branches, uneven shrinking of the stem above the collar region, which ultimately lead to collapse of chickpea seedlings, without discoloration in 20-30 days after sowing. When wilt affected seedlings were uprooted, the roots of the affected plant apparently appeared healthy. At adult stage, wilt symptoms in adult plants were quite common at flowering and pod stages. The affected plants showed characteristic wilting viz., drooping of petioles, rachis, and leaflets. The lower leaves were chlorotic, most of the leaves drooped while still green. Gradually, all the leaves become yellow and then light brown or straw coloured. Two types of wilt symptoms were observed viz., partial wilt and complete wilt. In case of complete wilt, all branches were affected on both side of plant. Drooped leaves later dried, but remained attached to the plant for long time. When stem of the wilted chickpea plant was split open longitudinally with a sharp-edged blade, internal was clearly distinctive which is quite characteristic of fusarium wilt. The xylem in the centre inner portion was discoloured as yellow to dark brown, pink to black.

Pathogenicity and virulence of *Fusarium oxysporum* f. sp. ciceri: *Fusarium oxysporum* f. sp. *ciceri* was tested for proving the pathogenicity of chickpea wilt. Pre-sterilized soil was inoculated with pure culture of *F. oxysporum* f. sp. *ciceri* isolates and seeds of susceptible chickpea variety C-235 were planted, regularly watered, and constantly observed from seedling emergence to development of wilt symptoms. The fungus produced initial symptoms on chickpea seedling after 15 days of inoculation under controlled conditions having characteristics drooping of petioles, rachis, and leaflets. The pathogen were re-isolated from collar and affected root region of plant was made and pathogenic

cultures obtained were compared with original culture of *Foc*. Prepared mass multiplied (*Fusarium oxysporum* f. sp. *ciceri*) on sterilized sorghum or wheat grains. The grains were softened by boiling in water for 20 minutes. Excess water was removed by draining also the grains were spread to cool down and decrease the moisture content. Calcium carbonate (2g) was added in 100g of pre-boiled semi-dried sorghum grains to remove excess moisture. The content was transferred to conical flasks and autoclaved at 15 lb/inch² pressure for 15 minutes. After cooling these flasks were inoculated with 4 discs of 5.0 mm diameter mycelial growth of three-day old culture of *F. oxysporum* f. sp. *ciceri* grown on PDA plate. The flasks were incubated at $28\pm 1^\circ\text{C}$ for seven days for colonization of sorghum grains. After proper colonization in the flasks, inoculum was mixed with sterilized soil @ 100g/kg soil and filled in the earthen pots (22.5cm diameter). The seeds of chickpea were moistened in sterilized water and simultaneously inoculated with powdered inoculum @ 5g/kg seed. These seeds were dried in shade and 10 seeds per pot were sown. Three replications per treatment were laid and an uninoculated control was also maintained. The plants were regularly watered and proper care was taken for maintenance of plants. Chickpea plants were regularly observed for the development of wilt symptoms properly recorded was maintained on daily basis and were recorded viz numerical rating of 0, 1, 2, 3, 4 and 5 on the basis of initial plant count and total number of wilted plants in each genotype (Nene *et. al.* 1981). The highly resistant plant having score of 0, while those with highest infection i.e. highly susceptible having score of 5.

RESULTS AND DISCUSSION

Status of wilt of chickpea in Jammu Division: Extensive fortnight surveys were undertaken during *Rabi* seasons 2016-17 and 2017-18, in major chickpea growing districts of Jammu division viz., Jammu, Samba and Kathua districts. During surveillance, certain location chickpea wilt having were overall range of 8.11-21.67 and 10.98-23.99 per cent respectively with an overall mean incidence of 15.64 and 16.86 per cent, respectively (Table 1). However, maximum disease incidence (21.67% and 23.99% respectively) was recorded in Chatha of Satwari block followed by Lehar of Akhnoor block, whereas, the lowest disease incidence was in Panotra Chak of Satwari block followed by Palori of Akhnoor. In Samba district, the highest disease incidence (20.76% and 21.98% respectively) was recorded in Dhiansar of Vijaypur block followed and Sumb of Samba block whereas lowest disease incidence was in Sanoora of Ghagwal block. In Kathua district, the average disease incidence ranged from 8.11 to 15.44 and 10.98-16.34 per cent respectively. The

Table 1. Area and source of isolation of *Fusarium oxysporum* f. sp. *ciceri*

Location	Isolate of <i>F. oxysporum</i> f. sp. <i>ciceri</i> (FOC)	Host variety	Growth habit
Jammu district			
Bishnah	FOC-1	C-235	Fast
	FOC-2	C-235	Slow
	FOC-3	C-235	Moderate
	FOC-4	C-235	Poor
	FOC-5	C-235	Poor
	FOC-6	C-235	Slow
	FOC-7	C-235	Poor
Jammu	FOC-8	Local cultivar	Moderate
	FOC-9	Local cultivar	Slow
	FOC-10	Local cultivar	Poor
	FOC-11	Local cultivar	Slow
	FOC-12	Local cultivar	Poor
	FOC-13	Local cultivar	Slow
Sungal	FOC-14	VBN-6	Moderate
	FOC-15	VBN-6	Poor
	FOC-16	VBN-8	Slow
	FOC-17	VBN-8	Moderate
	FOC-18	VBN-8	Fast
	FOC-19	Local cultivar	Moderate
Samba district	FOC-20	Local cultivar	Moderate
	Vijaypur		
Samba	FOC-21	PBG-1	Poor
	FOC-22	PBG-1	Slow
	FOC-23	PBG-1	Slow
	FOC-24	PBG-1	Slow
	FOC-25	PBG-1	Moderate
	FOC-26	SCS-3	Poor
	FOC-27	SCS-3	Poor
	FOC-28	SCS-3	Fast
	FOC-29	SCS-3	Poor
	FOC-30	SCS-3	Slow
	FOC-31	SCS-3	Poor
Ghagwal	FOC-32	GNG 496	Slow
	FOC-33	GNG 496	Slow
	FOC-34	GNG 496	Fast
	FOC-35	GNG 496	Slow
	FOC-36	GNG 496	Slow
Kathua district			
Hiranagar	FOC-37	GNG 496	Fast
	FOC-38	GNG 496	Fast
	FOC-39	GNG 496	Moderate
	FOC-40	GNG 496	Moderate
	FOC-41	GNG 496	Fast
Barnoti	FOC-42	Local Variety	Slow
	FOC-43	Local Variety	Fast
	FOC-44	Local Variety	Slow
	FOC-45	Local Variety	Poor
	FOC-46	Local Variety	Poor
Kathua	FOC-47	Gaurav	Slow
	FOC-48	Gaurav	Moderate
	FOC-49	Gaurav	Slow
	FOC-50	Gaurav	Slow

Table 2. Status of chickpea wilt in Jammu sub-tropics during *Rabi* 2016-17 and 2017-18

Block	Location	Disease incidence (%)		
		2016-17	2017-18	Pooled
Jammu district				
Bishnah	Deoli	15.32	16.78	16.04
	Dhabad	20.18	20.97	20.57
	Pataidi	17.98	18.87	18.42
	Mean ± S.E.	17.82± 1.93	18.87 ± 1.72	18.34± 0.05
Satwari	Panotra Chak	12.98	14.87	13.92
	Sohanjna	18.89	19.98	19.43
	Chatha	21.67	23.99	22.83
	Mean ± S.E.	17.84 ± 2.56	19.61 ± 2.63	18.72± 0.18
Akhnoor	Lehar	20.76	21.54	21.15
	Palori	14.87	15.98	15.42
	Pangiari	17.11	18.99	18.04
	Mean ± S.E.	17.58 ± 1.72	18.83 ± 1.60	18.20± 0.05
Samba district				
Vijaypur	Chhanni	17.90	18.94	18.41
	Dhiansar	20.76	21.98	21.36
	Salmeri	15.87	16.10	15.98
	Mean ± S.E.	18.17 ± 1.42	19.00 ± 1.70	18.58± 0.41
Samba	Nanke	16.98	17.98	17.47
	Sumb	18.77	19.47	19.11
	Rakh	15.54	16.09	15.81
	Mean ± S.E.	17.09 ± 0.93	17.84 ± 0.97	17.46± 0.02
Ghagwal	Ragu Chak	19.98	20.98	20.35
	Sanoora	11.46	12.89	12.17
	Harsat	15.99	16.67	16.33
	Mean ± S.E.	15.81 ± 2.46	16.84 ± 2.33	16.28± 0.00
Kathua district				
Hiranagar	Kootah	8.11	10.98	9.54
	Jatwal	10.67	12.87	11.76
	Tokal	11.32	12.76	12.03
	Mean ± S.E.	10.03 ± 0.98	12.20 ± 0.61	11.11± 0.04
Barnoti	Budhi	11.87	12.65	12.25
	Sonthal	13.45	14.98	14.21
	ChhannRorian	15.44	16.34	15.88
	Mean ± S.E.	13.58 ± 1.03	14.65 ± 1.07	14.11± 0.03
Kathua	Rajbagh	14.78	15.54	15.15
	Lagate	12.45	13.67	13.05
	Hatli	11.45	12.67	12.05
	Mean ± S.E.	12.89 ± 0.98	13.96 ± 0.84	13.41± 0.00
	Overall mean	15.64	16.86	16.25
	Overall range	8.11 – 21.67	10.98 – 23.99	9.54-22.83

highest disease incidence was recorded in Chhann Rorian of Barnoti block followed by Rajbagh of Kathua block. Many workers have reported chickpea wilt throughout the crop season with maximum incidence and damage in the chickpea crop at seedling stage than at maturity. Disease was prevalent in all the chickpea growing areas surveyed. The wilt symptoms at seedling stage were observed about 2-3 weeks after sowing in the first fortnight of November. The affected seedlings retained almost green colour. The whole lot of chickpea seedling collapsed and laid flat on the ground. Such collapsed seedlings, when uprooted usually showed uneven shrinking of the stem above and below the collar region. Upon dissection, collar region showed black discoloration of internal tissues. The wilt affected mature plants showed typical wilting accompanied by drooping of petioles and rachis along with leaflets.

Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri* isolates on susceptible chickpea cultivar (C-235): The pathogenicity test of *F. oxysporum* f. sp. *ciceri* (Foc) isolate causing chickpea wilt was determined on susceptible chickpea cultivar (C-235) grown in sterilized pot soil at seedling stage. Data (Table 2) revealed that chickpea wilt incidence ranged between 6.26-66.65 per cent. However, eight isolates of the wilt pathogen viz., FOC-1, FOC-18, FOC-28, FOC-34, FOC-37, FOC-38, FOC-41 and FOC-43 exhibited excellent virulence pattern and showed symptoms 15 days after sowing; 10 isolates exhibited good virulence pattern and wilt symptoms expressed in 21 days after sowing; 19 isolates recorded fair virulence pattern and 13 isolates exhibited poor virulence pattern and the disease symptoms were visible 32 days after sowing. None of the Foc isolate was avirulent. Each inoculated isolate of Foc was re-isolated from the infected chickpea plants and compared with the original culture. Morphological characters of the isolated pathogen were found to be similar to those of the original culture. Hence, it was confirmed that the fungus *F. oxysporum* f. sp. *ciceri* was pathogenic and responsible for causing wilt of chickpea, thereby confirming the Koch's postulates. However, no symptoms developed on uninoculated chickpea plants throughout the crop season. The findings are also in consonance with the studies of Patra and Biswas (2016) on cultural, morphological and pathogenic variability among the 11 isolates of *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea who observed that most of the isolates were highly pathogenic. Patil et al (2017) proved the pathogenicity of *F. oxysporum* f. sp. *ciceri* isolates by using highly susceptible chickpea variety JG-62 and resistant variety JG-315 and reported that initiation of disease due to different isolates ranged from 5-10 days depending upon susceptibility of the host.

Table 3. Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri* isolates on susceptible chickpea cultivar (C-235)

Isolate	Location	Disease incidence (%)	*Virulence pattern
Jammu district			
FOC-1	Bishnah	66.65	++++
FOC-2		26.26	++
FOC-3		33.13	+++
FOC-4		13.39	+
FOC-5		6.86	+
FOC-6		20.00	++
FOC-7		6.56	+
FOC-8	Satwari	33.73	+++
FOC-9		20.60	++
FOC-10		13.43	+
FOC-11		26.96	++
FOC-12		13.73	+
FOC-13		26.66	++
FOC-14	Sungal	33.83	+++
FOC-15		13.23	+
FOC-16		20.50	++
FOC-17		33.73	+++
FOC-18		46.76	++++
FOC-19		33.83	+++
FOC-20		33.93	+++
Samba district			
FOC-21	Vijaypur	6.26	+
FOC-22		26.06	++
FOC-23		20.50	++
FOC-24		20.09	++
FOC-25		33.37	+++
FOC-26	Samba	13.34	+
FOC-27		13.63	+
FOC-28		46.86	++++
FOC-29		13.73	+
FOC-30		20.70	++
FOC-31		13.93	+
FOC-32	Ghagwal	26.36	++
FOC-33		26.96	++
FOC-34		40.40	++++
FOC-35		26.76	++
FOC-36		26.86	++
Kathua district			
FOC-37	Hiranagar	40.60	++++
FOC-38		46.46	++++
FOC-39		33.93	+++
FOC-40		33.93	+++
FOC-41		40.50	++++
FOC-42	Barnoti	26.86	++
FOC-43		40.90	++++
FOC-44		26.86	++
FOC-45		6.76	+
FOC-46		13.53	+
FOC-47	Kathua	26.96	++
FOC-48		33.53	+++
FOC-49		20.80	++
FOC-50		26.56	++

*Absent Avirulent
+ Poor (Symptoms appeared 32 DAS)
++ Fair (Symptoms appeared 28 DAS)
+++ Good (Symptoms appeared 21 DAS)
++++ Excellent (Symptoms appeared 15 DAS)

Table 4. Sporulation of *Fusarium oxysporum* f. sp. *ciceri* isolates on PDA

Isolate	Spore density [No. of spores/ microscopic field (10x)]			Total no. of spores	Grade
	Microconidia	Macroconidia	Chlamydospore		
FOC-1	34	23	11	68	++++
FOC-2	20	11	4	35	++
FOC-3	29	18	7	54	+++
FOC-4	12	7	3	22	+
FOC-5	9	5	1	15	+
FOC-6	20	13	4	37	++
FOC-7	11	5	1	17	+
FOC-8	29	19	2	51	+++
FOC-9	18	12	2	32	++
FOC-10	13	6	4	23	+
FOC-11	25	14	3	42	++
FOC-12	25	11	2	38	++
FOC-13	24	14	2	40	++
FOC-14	20	11	4	35	++
FOC-15	17	12	3	32	++
FOC-16	23	12	3	38	++
FOC-17	20	13	1	34	++
FOC-18	31	24	7	62	++++
FOC-19	26	19	7	52	+++
FOC-20	24	17	6	47	+++
FOC-21	13	9	1	25	+
FOC-22	17	11	4	32	++
FOC-23	22	10	5	37	++
FOC-24	25	14	3	42	++
FOC-25	27	17	2	46	+++
FOC-26	15	7	3	25	+
FOC-27	12	6	4	22	+
FOC-28	31	25	6	62	++++
FOC-29	15	9	4	28	+
FOC-30	22	11	2	35	++
FOC-31	13	8	3	24	+
FOC-32	24	12	1	37	++
FOC-33	22	14	3	39	++
FOC-34	29	19	2	50	+++
FOC-35	20	11	3	34	++
FOC-36	22	9	3	34	++
FOC-37	19	11	2	32	++
FOC-38	31	21	6	58	+++
FOC-39	29	22	4	55	+++
FOC-40	28	19	5	52	+++
FOC-41	21	14	2	37	++
FOC-42	24	16	1	41	++
FOC-43	29	20	2	51	+++
FOC-44	25	16	3	44	++
FOC-45	14	6	0	20	+
FOC-46	11	7	4	22	+
FOC-47	24	11	3	38	++
FOC-48	28	17	3	48	+++
FOC-49	21	14	4	39	++
FOC-50	21	14	3	38	++
CD (p=0.05)				2.98	

Grade	Sporulation	Spores/ microscopic field
++++	Very good	>60
+++	Good	45.1-60
++	Moderate	30.1-45
+	Poor	<30

Sporulation of *Fusarium oxysporum* f. sp. *ciceri* isolate on PDA: The growth of *F. oxysporum* f. sp. *ciceri* (Foc) was drastically reduced below 15°C and started declining above 35°C as higher temperatures did not favour growth of the fungus. Best growth of the pathogenic fungus was recorded between 25-30°C. The results (Table 3) revealed that all the fifty isolates of Foc tested during the present studies exhibited good fungal growth and sporulation on PDA culture medium. The spore density of microconidia, macroconidia and chlamydospores, however, varied from in different isolate of the fungus.

With respect to microconidia, maximum spore density of 34 was observed in FOC-1 followed by 31 in FOC-18, FOC-28 and FOC-38, whereas minimum spore density of 9 was in FOC-5. With respect to macroconidia, maximum spore density of 25 was in FOC-28 followed by 24 in FOC-18; 23 in FOC-1 and 22 in FOC-39, whereas minimum spore density of 5 was in FOC-5 and FOC-7. With respect to chlamydospore, maximum spore density of 11 was observed in FOC-1 followed by FOC-3, FOC-18 and FOC-19; whereas minimum spore density of 1 in FOC-5, FOC-7, FOC-17, FOC-21, FOC-32 and FOC-42. Three isolates viz., FOC-1, FOC-18 and FOC-28 were having very good sporulation with >60 spores/microscopic field; 11 isolate viz., FOC-3, FOC-8, FOC-19, FOC-20, FOC-25, FOC-34, FOC-38, FOC-39, FOC-40, FOC-43 and FOC-48 had good sporulation with 45.1-60 spores/microscopic field; 25 isolate were having moderate sporulation with 30.1-45 spores/microscopic field and 11 isolates recorded poor sporulation with <30 spores/microscopic field. Nath et al (2017) also observed that different isolates of *F. oxysporum* f. sp. *ciceri* infecting chickpea were collected from major chickpea growing areas of Bangladesh and varied significantly in cultural, morphological and physiological traits, i.e. colony color, shape, margin and texture; mycelial radial growth and spore production. The mycelial radial growth and sporulation of *F. oxysporum* was maximum for all the isolates at 25°C after seven days of inoculation, which was reduced drastically below 15°C and above 35°C. The highest number of macro spores ($3.27 \times 10^5 \text{ ml}^{-1}$) and micro spores ($4.06 \times 10^5 \text{ ml}^{-1}$) were produced on PDA.

CONCLUSION

Wilt symptoms in adult plants were abundant at flowering and pod stages. Fortnightly surveys undertaken during *Rabi* seasons of 2016-17 and 2017-18 revealed that wilt disease appeared on chickpea crop at all the locations and overall

range of disease incidence in 2016-17 and 2017-18. Based on pathogenicity, Foc isolates causing chickpea wilt was confirmed on the susceptible chickpea cultivar (C-235) grown in sterilized pot soil at seedling stage. All the fifty isolates of Foc exhibited good fungal growth and sporulation on PDA culture medium where Maximum number of spores 68 total was observed in FOC-1, while minimum number of spores 15 was observed in FOC-5.

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